

Serial No. 09/120,044  
Docket No. 1758-4036US2

position 172, and wherein at least one of said amino acid substitutions results in attenuation of the hemolytic activity of the modified pneumolysin polypeptide, and wherein said modified pneumolysin polypeptide is obtained by:

*E  
C  
A*

- a) randomly mutating a nucleic acid molecule encoding type 14 pneumolysin polypeptide to produce a mutated nucleic acid molecule encoding modified pneumolysin polypeptide and expressing the mutated nucleic acid molecule in a host cell;
- b) assaying the modified polypeptide expressed by the host cell for hemolytic activity; and
- c) identifying a modified refoldable pneumolysin polypeptide having similar molecular weight as native type 14 pneumolysin.

2. (twice amended) A modified refoldable pneumolysin polypeptide having attenuated hemolytic activity comprising an amino acid sequence of type 14 pneumolysin wherein at least one amino acid in the region comprising amino acids 1 to 257 (SEQ ID NO: 3)[, and besides a substitution of threonine for isoleucine at amino acid 172,] is substituted, with the proviso that the substitution is not solely a substitution of isoleucine for threonine at position 172, and wherein at least one of said amino acid substitution results in attenuation of the hemolytic activity of the modified pneumolysin polypeptide.

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#### REMARKS

Support for the claim amendments is found throughout the specification; for example, at page 5, line 28 through page 6, line 3, and in Table 5A which indicates that threonine is the native amino acid at position 172. No new matter is added to the application by these claim amendments. Further, the amendments raise no new issues that require further reconsideration or search. Applicants' amendments were not previously filed because the amendments are made in response to a rejection